Abstract No. kato61

Crystal Structure of the Primase Domain of the T7 Primase-Helicase

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Beamline(s): X12B, X12C, X8C, and X25

Introduction: The gene 4 primase-helicase of bacteriophage T7 plays a central role in the T7 replication fork, coordinating both leading and lagging strand DNA synthesis. For leading strand synthesis, the primase-helicase unwinds double strand DNA while tightly coupled to the leading strand DNA polymerase. For lagging strand synthesis, the primase-helicase synthesizes tetraribonucleotide primers de novo then transfers the primer to the active site of the lagging strand polymerase in a transient complex. These primase and helicase activities reside in the N- and C-terminal domains of the primase-helicase, respectively, and also these domains are still active even if separated form each other. The crystal structures of the helicase domain of the T7 primase-helicase have already been determined, and they have revealed a possible mechanism of unwinding dsDNA [1,2]. On the other hand, although the DNA binding and catalytic properties of the T7 primase are well characterized, the structure of the primase domain has not been solved yet. Here, we determined the structure to address the mechanism of the primer synthesis.

Methods and Materials: The primase domain (residue 1–255) was crystallized in the condition of 50 mM MES (pH 6.3), 4 M Sodium Formate, 5 mM DTT, 2.5 mM $MgCl_2$, and 2.5 mM ATP. The crystals grow up to the maximum size of 0.8 * 0.4 * 0.4 mm^3 in a week and diffract to 2.3 Å at synchrotron. They belong to the space group of $P3_121$ and contain two molecules in the asymmetric unit. The experimental phases were determined by MAD using the crystal of Se-met substituted protein. Eleven of the total 14 Se sites were found by SOLVE and refined by MLPHARE. The phases were further improved by DM with averaging. The electron density map was so clear that the entire backbone could be traced. The current model, including 494 amino acids and two zinc ions, was refined with CNS to an R-factor of 32% (free R-factor 35%).

Results: The primase domain has two distinct domains, N-terminal zinc finger domain and C-terminal catalytic core domain (Figure 1). The zinc finger domain is far from the core domain connected with a long arm region. Although there is no evidence of dimerization in solution, the primase domain forms a dimer in the crystal. The arm region in each subunit intersects each other, and the N-terminal domain seems to be swapped between two subunits. The catalytic core domain showed the structure similarity with that of *Escherichia coli* DnaG [3], even though the sequence identity is less than 20%. The potential catalytic site of primer synthesis is formed by the conserved acidic residues located in the cleft. A model refinement is further in progress.

References:

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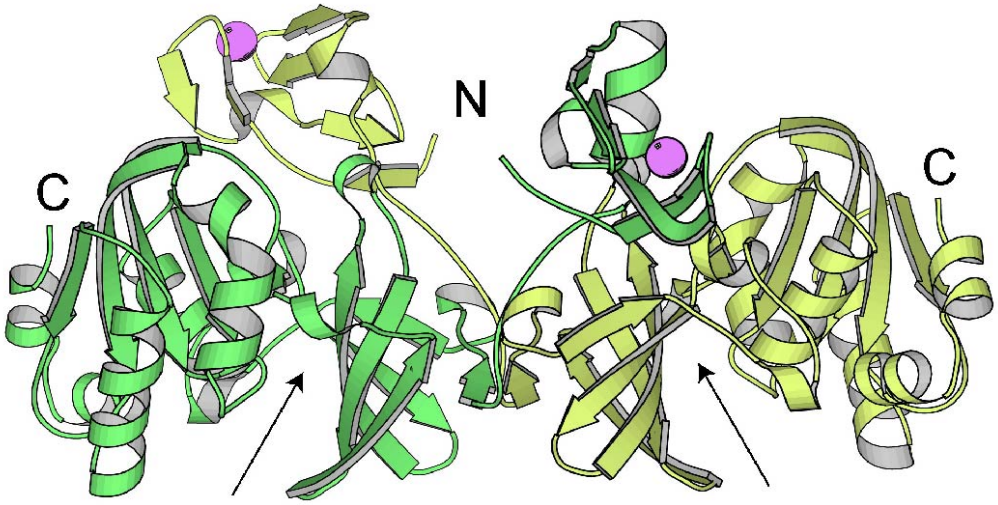

Figure 1. The structure of T7 primase domain. Each subunit is represented with green and yellow, respectively. Zinc atoms are depicted purple balls. Allows indicate the cleft of the potential catalytic site.